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Keywords

Diabrotica barberi, *Diabrotica virgifera virgifera*, Bt Corn, Insect Resistance Management, Integrated Pest Management

Disciplines

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Dunbar et al.: Corn Rootworm and Cropping History

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Abstract

Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), and northern corn rootworm, *Diabrotica barberi* Smith & Lawrence, are major pests of corn (*Zea mays* L.). Corn producing *Bacillus thuringiensis* (Bt) toxins are widely used to manage *Diabrotica* spp.; however, Bt resistance by *D. v. virgifera* has led to high levels of feeding injury in the field. We tested whether field history affected root injury and abundance of adult *Diabrotica* spp. In 2013 and 2014, four types of cornfields were sampled: 1) recently rotated fields, 2) continuous cornfields, 3) fields with a history of injury to Bt corn (past problem fields) and 4) fields with greater than one node of injury to Bt corn at the time of sampling (current problem fields). Data were collected on field history, root injury and the abundance of adult *Diabrotica* spp. from each field. Root injury and the abundance of *D. v. virgifera* were significantly greater in current problem fields compared to the other field types, while *D. barberi* were significantly more abundant in recently rotated fields. Root injury and the abundance of *D. v. virgifera* did not differ among recently rotated fields, continuous cornfields and past problem fields. Analysis of field history showed that recently rotated fields were characterized by significantly less Bt corn, soil-applied insecticides and years planted to corn continuously. These results suggest that greater cropping practice diversity can reduce management inputs for *Diabrotica* spp.; however, its effects on resistance evolution remain undetermined.

Key Words: *Diabrotica barberi*, *Diabrotica virgifera virgifera*, Bt Corn, Insect Resistance Management, Integrated Pest Management

Introduction

Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae), western corn rootworm, and *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), northern corn rootworm, are important pests of corn (*Zea mays* L.) in North America. Both species are univoltine with life cycles tightly linked to corn (Chiang 1973, Meinke et al. 2009, Spencer et al. 2009). Adult *D. barberi* and *D. v. virgifera* feed on corn silks, pollen, and kernels, although *D. barberi* will readily consume plant material from other species (Lance and Fisher 1987). Eggs are predominantly oviposited in the soil of cornfields, diapause through the winter and larvae hatch during the spring. Larvae of both species feed on corn roots and the resulting injury reduces the ability of plants to take up water and nutrients (Levine and Oloumi-Sadeghi 1991). Larval feeding on corn roots is the primary cause of yield loss from *Diabrotica* spp. (Gray et al. 2009). Corn yield is reduced by 15 to 17% for each node of root injury (Dun et al. 2010, Tinsley et al. 2013).

Farmers have several management options to mitigate injury from *Diabrotica* spp. Crop rotation has been used to manage *Diabrotica* spp. for over a century (Spencer et al. 2014), and is effective because larvae that hatch in fields rotated to a non-corn crop starve (Branson and Ortman 1970, 1971). However, in parts of the Corn Belt, both *D. v. virgifera* and *D. barberi* have evolved resistance to crop rotation; *D. v. virgifera* adapted behaviorally through a loss of host plant ovipositional fidelity (Levine et al. 2002) and *D. barberi* adapted physiologically with variable length, extended diapause (Krysan et al. 1984). Conventional insecticides have been used for management of *Diabrotica* spp. since the 1940s, and can either be applied to the soil at planting to reduce larval feeding on roots or aerially to both decrease silk feeding and suppress adult populations and oviposition (Levine and Oloumi-Sadeghi 1991). Since 2003, genetically

modified corn producing insecticidal toxins derived from *Bacillus thuringiensis* (Bt) has been commercially available to manage larval *Diabrotica* spp. (EPA 2003). There are currently four *Diabrotica*-active Bt toxins available, produced in corn either singly or as a pyramid: Cry3Bb1, Cry34/35Ab1, mCry3A and eCry3.1Ab (Cullen et al. 2013).

Bt corn has been widely used by farmers for insect pest management. Rapid adoption of Bt crops has led to concerns over the development of resistant populations (Gould 1998, Onstad et al. 2001, Carrière et al. 2010). Seventy-two percent of corn planted in the U.S. during 2015 produced Bt toxins that targeted pest insects (USDA, NASS 2015), including *D. v. virgifera* and *D. barberi*. Within seven years of commercialization of Bt corn targeting *Diabrotica* spp., cornfields in Iowa were observed with severe root injury associated with field-evolved resistance to Cry3Bb1 by *D. v. virgifera* (Gassmann et al. 2011). More reports of Cry3Bb1 resistance in Iowa, Illinois and Nebraska were soon published (Gassmann et al. 2012, 2014; Gray 2014, Wangila et al. 2015) as well as cross-resistance between Cry3Bb1 and mCry3A toxins (Gassmann et al. 2014, Wangila et al. 2015). At present, there are no reports of field-evolved resistance by *D. v. virgifera* to either Cry34/35Ab1 (Gassmann et al. 2014) or eCry3.1Ab.

Integrated Pest Management (IPM) combines multiple management strategies with knowledge of pest ecology in order to sustainably manage pest populations (Stern et al. 1959). Compared to monocultures, agroecosystems with greater vegetational diversity have been associated with decreased pest recruitment and retention (Andow 1991, Landis et al. 2000), particularly for pests with narrow host ranges (Root 1973). Studies have shown that increasing the diversity or length of crop rotations can reduce reliance on chemical inputs such as pesticides while protecting crop yields (Bullock 1992, Smith et al. 2008, Davis et al. 2012). Additionally,

IPM can decrease the intensity of selection pressure on pest populations to evolve resistance to any single strategy by diversifying the causes of pest mortality (McGaughey and Whalon 1992).

As populations of Bt-resistant *D. v. virgifera* continue to develop, pest-management may require more than one tactic to meet this new challenge. Fields with Bt-resistant *D. v. virgifera* were typically characterized by continuous planting of corn dominated by hybrids expressing Cry3Bb1 (Gassmann et al. 2011, 2012; Wangila et al. 2015). Integrating alternate management tactics for *D. v. virgifera*, such as crop rotation or conventional insecticides, in coordination with Bt crops, may mitigate the risk of injury from Cry3Bb1 and mCry3A-resistant *D. v. virgifera* and sustain the efficacy of the remaining Bt toxins (Gassmann 2012, Tabashnik and Gould 2012). Our objectives were to test the effects of field history, including crop rotation and management of *Diabrotica* spp., on root injury and abundance of adult *D. v. virgifera* and *D. barberi*. During 2013 and 2014, we sampled 47 cornfields in Iowa that were either recently rotated (second-year cornfields), planted to long-term continuous corn (> seven years of continuous corn), had previously experienced greater than one node of injury to Bt corn from *D. v. virgifera* or were currently experiencing severe *Diabrotica* spp. injury to Bt corn. We hypothesized that root injury and abundance of adult *Diabrotica* spp. would be reduced in fields with more frequent crop rotation and additional pest management practices compared to fields that lack diverse cropping history. Additionally, we compared how farmers mitigated Bt resistance in cornfields that had previously experienced severe injury to either Cry3Bb1 or mCry3A corn. We hypothesized that farmers would mitigate management challenges from *D. v. virgifera* by either rotating fields away from corn, changing the variety of Bt corn planted, applying more conventional insecticides or some combination of these factors.

Materials and Methods

Field Selection. In 2013 and 2014, cornfields in Iowa were sampled to measure root injury from larval *Diabrotica* spp. and the abundance of adult *D. barberi* and *D. v. virgifera* (Fig. 1). Fields were identified by Iowa State University regional agronomists and local cooperators. The fields sampled fit into one of four types: 1) recently rotated fields that were planted to corn for the second consecutive year at the time of a sampling, 2) continuous cornfields were fields planted to corn for a minimum of seven consecutive years, 3) past problem cornfields were fields that had been previously sampled for root injury and had greater than expected injury (> 1 node of root injury (EPA 2011)) to either Cry3Bb1 corn ($n = 11$) or mCry3A corn ($n = 2$), as measured by the 0 to 3 root-node injury scale (Oleson et al. 2005), and had been planted to corn for at least two years at the time of sampling, and 4) current problem fields that were fields with greater than expected injury to Bt corn (> 1 node of root injury) during the year the field was sampled, which was either 2013 or 2014. Cooperating farmers identified recently rotated fields and continuous cornfields based solely on cropping history (i.e., 2nd-year or > 7 -year cornfields), independent of previous *Diabrotica* spp. injury or pest management practice. Past problem fields were initially sampled between 2009 and 2013, and roots sampled from these fields (10.8 ± 1.2 roots per field, mean \pm SEM) scored on average 2.1 ± 1.2 nodes of roots injured (mean injury score per field ranged from 1.56 to 2.91).

Data Collection. Farmers were asked to provide a history of the crops planted and management practices used for *Diabrotica* spp. in each field. When possible, these field histories were collected starting from the year sampled through 2003 (the commercial introduction of Bt corn targeting *Diabrotica* spp.). Data collected included the crop planted each year, and for years

when corn was planted, whether a Bt trait was planted (if yes, which trait) and if a soil or foliar-applied insecticide was used to manage *Diabrotica* spp.

Adult *Diabrotica* spp. were sampled from cornfields with unbaited Pherocon AM yellow sticky traps (referred to as sticky traps; Trécé Inc., Adair, OK) using methods similar to Dunbar and Gassmann (2013). Twelve sticky traps were placed within each cornfield and arranged in two transects. Transects were separated by a minimum of 100 m and kept 50 m from any field edge. Sticky traps were stationed at 20 m intervals along each transect and were attached to corn plants at ear height. Traps were placed in cornfields throughout the first three weeks of August and were changed weekly throughout this period (sampled weeks began 6 Aug., 13 Aug., and 20 Aug. during 2013 and 4 Aug., 11 Aug., and 18 Aug. during 2014).

Twelve roots were sampled from the interior of each cornfield during August (during the week of 20 Aug. in 2013 and the week of 18 Aug. 2014). Roots were sampled along the same transects that were used for sticky traps. Corn roots were sampled from plants 1.5 m distant from the corn plant holding a sticky trap. Mean root injury was quantified based on the 0 to 3 node-injury scale (Oleson et al. 2005) from all 12 roots sampled. Some fields visited as current problem fields had mean node-injury scores of less than 1 node scores of less than 1 node. Fields with less than 1 node of injured corn root were excluded from further analyses (4 of 9 fields in 2013 and 1 of 5 fields in 2014). Corn roots were tested for the presence of *Diabrotica* spp. active Bt toxin with an ELISA kit (Envirologix, Portland, ME). In 2013, all 12 roots from each field were tested for Cry3Bb1, Cry34/35Ab1 and mCry3A toxins. In 2014, the presence of each Bt toxin (Cry3Bb1, Cry34/35Ab1, mCry3A and eCry3.1Ab) was tested from a random subset of corn roots (mean subset = 6.7 per Bt toxin, SEM = 0.16).

Data Analysis. For each field and each week sampled, the total number of *D. barberi* and *D. v. virgifera* captured by each sticky trap was counted. The total number of each species was then averaged among the 12 sticky traps placed within each field for a sampling week. The average *D. barberi* and *D. v. virgifera* captured per sticky trap per sampling week was divided by the number of days sticky traps were in the field to calculate the average species captured per sticky trap per day for each week sampled. Data from the week with the highest capture of *D. barberi* and *D. v. virgifera* captured per sticky trap per day, which we defined as peak abundance, was used for all analyses.

Root injury, peak abundance of *D. v. virgifera* and peak abundance of *D. barberi* were analyzed separately per year with mixed-model analysis of variance (ANOVA) using PROC MIXED in SAS 9.3 (SAS Institute, Cary, NC). To ensure normality of the residuals, *D. barberi* and *D. v. virgifera* data were transformed by the function square root (x) and root injury data by the function log (x). In the analysis, field type was a fixed effect and random effects included 1) location nested within field type and 2) sticky trap or root sampled nested within location \times field type. Additionally for each field, the ratio of *D. v. virgifera* to *D. barberi* was calculated by dividing the total number of *D. v. virgifera* by the total number of *D. barberi* captured from all sticky traps from all weeks sampled. Ratios were compared among field types, separately by year, using mixed-model ANOVA (PROC MIXED). Data were transformed by the function log (x) to ensure normality of the residuals. Field type was a fixed effect in the analysis and location nested within field type was the random effect. When significant effects were present in a model, pairwise comparisons were made using the PDIFF option in PROC MIXED and alpha levels were adjusted for multiple comparisons using the Bonferroni correction.

To compare the field history data among field types, we used the proportion of years a field was planted to corn, planted to corn continuously (i.e., the number of years in which corn was grown for two or more years consecutively) and received other management practices for *Diabrotica* spp. (i.e., Cry3Bb1 corn or soil-applied insecticide). Fields where less than 75% of the field history was known were excluded from further analyses (n = 10 fields excluded) (Supp. Tables S1 - S4). The percentage of field history known for each field was calculated as the number of complete years of field history / the total number of years (i.e., 11 years for fields sampled in 2013 and 12 years for fields sampled during 2014). When field histories were not complete, crops planted within fields for all unknown years were identified using CropScape - Cropland Data Layer (USDA, NASS 2016). A year where a field was planted to corn was complete if the type of Bt corn planted and the use of soil and foliar-applied insecticide were known. Years could also be scored as partially complete (e.g., unknown Bt corn, known use of soil insecticide, known use of foliar insecticide = a 0.66 complete year), and this was counted in the calculation of whether or not field history was 75% complete. A year where a field was planted to a known non-corn crop was viewed as complete. For fields included in the analysis that had years with missing management data, management tactics were assumed to occur with the same frequency in years with missing data as they did for years which a field history was known.

Multiple regression analysis (PROC REG) was used to test the effect of field history on root injury, peak abundance of *D. v. virgifera*, peak abundance of *D. barberi* and the ratio of *D. v. virgifera* to *D. barberi*. Root injury and adult *Diabrotica* spp. data from 2013 and 2014 were combined and transformed by the function $\log(x)$. Bi-directional elimination (SELECTION = STEPWISE) was used for excluding ($P > 0.15$) and including ($P < 0.15$) variables in the model

(Littell et al. 1996). Factors used in regression analyses included each field type, the management practices used for *Diabrotica* spp. during the year a field was sampled (i.e., non-Bt, Cry3Bb1, Cry34/35Ab1, mCry3A or Cry3Bb1 + Cry34/35Ab1 corn; application of soil or foliar insecticide), and the proportions of years each field was: 1) planted to corn, 2) planted corn continuously, 3) planted to a specific Bt corn (Cry3Bb1, Cry34/35Ab1, mCry3A or Cry3Bb1 + Cry34/35Ab1), 4) planted to non-Bt corn, 5) treated with soil-applied insecticide and 6) treated with foliar-applied insecticide.

Field histories were compared among field types with mixed-model ANOVA (PROC MIXED). Factors tested were the proportions of years a field was: 1) planted to corn, 2) planted to corn continuously, 3) planted to non-Bt corn, 4) planted to a specific type of Bt corn (Cry3Bb1, Cry34/35Ab1, mCry3A or Cry3Bb1 + Cry34/35Ab1), 5) treated with soil-applied insecticide and 6) treated with foliar-applied insecticide. For the analysis of field histories among field types, field type was a fixed effect and the random effect was location nested within field type. When significant effects were present, pairwise comparisons were made using the PDIF option in PROC MIXED and alpha levels were adjusted for multiple comparisons using the Bonferroni correction. Additional analyses were conducted separately to compare field histories of past problem fields between the time before and after greater than expected injury to Bt corn was observed. Factors tested were the same as the analysis of field histories compared among field types. Frequency of use for each management tactic was compared before and after greater than expected injury with a paired two-tailed *t*-test (PROC TTEST).

Results

Field Sites. Of the 47 cornfields sampled in 2013 and 2014, 89% were planted to Bt corn (Table 1). Most cornfields were planted to pyramided Bt corn producing Cry3Bb1 and Cry34/35Ab1 toxins (47%), followed by Cry3Bb1 corn (21%), Cry34/35Ab1 corn (15%), non-Bt corn (11%) and mCry3A corn (6%). Soil insecticide was applied to 25% of all cornfields, of which 83% were also planted to Bt corn. Eleven of the 13 past problem fields were planted to pyramided corn, and soil insecticide was applied to 45% those pyramided Bt cornfields. Current problem fields were all planted to single trait Bt corn, expressing either Cry3Bb1 (78%) or mCry3A (22%), without soil insecticide.

Root Injury. Root injury differed significantly among field types in both 2013 and 2014 (Fig. 2). In 2013, current problem fields had significantly greater feeding injury to roots compared to all other field types ($F = 12.4$; $df = 3, 16$; $P = 0.0002$); root injury in current problem fields averaged over two nodes destroyed by larval feeding. The pattern of root injury among field types was similar in 2014. Current problem fields had significantly more root injury compared to the other field types ($F = 26.2$; $df = 3, 23$; $P < 0.0001$). Roots from recently rotated fields, continuous cornfields and past problem fields had very little injury and did not differ significantly from each other in either year.

Adult Abundance. Peak abundance of *D. v. virgifera* in 2013 was significantly greater in current problem fields compared to all other field types ($F = 18.8$; $df = 3, 16$; $P < 0.0001$) (Fig. 3). Within recently rotated fields, continuous cornfields and past problem fields, peak abundance of *D. v. virgifera* did not differ. In 2014, peak abundance of *D. v. virgifera* was significantly greater in current problem fields compared to the other field types ($F = 9.0$; $df = 3, 23$; $P =$

0.0004) (Fig. 3), with no significant differences among recently rotated fields, continuous cornfields and past problem fields.

Abundance of *D. barberi* was generally lower than that of *D. v. virgifera* during both years sampled. Peak abundance of *D. barberi* differed significantly among field types in 2013 ($F = 6.1$; $df = 3, 16$; $P = 0.006$) (Fig. 4). Recently rotated fields and continuous cornfields had significantly greater peak abundance of *D. barberi* compared to current problem fields, and peak abundance in past problem fields did not differ from any other field type. Peak abundance of *D. barberi* did not differ among field types in 2014 ($F = 0.6$; $df = 3, 23$; $P = 0.62$) (Fig. 4).

The ratio of *D. v. virgifera* to *D. barberi* within cornfields differed significantly among field types in 2013 ($F = 9.5$; $df = 3, 16$; $P = 0.0008$) and in 2014 ($F = 5.1$ $df = 3, 23$; $P = 0.007$). In 2013, current problem fields had a significantly greater ratio of *D. v. virgifera* to *D. barberi* than the other field types, but no differences were detected among recently rotated fields, continuous cornfields and past problem fields (Table 2). In 2014, current problem fields had a significantly greater ratio of *D. v. virgifera* to *D. barberi* than recently rotated fields, while continuous cornfields and past problem fields were intermediate and did not differ significantly (Table 2).

Field History. Multiple regression analysis of field history explained 59% of the observed variation in root injury (Table 3). The only parameter retained in the model was current problem fields, which was positively correlated with root injury. Multiple regression analysis explained 51% of the variation in the abundance of *D. v. virgifera*, and the model retained the parameters of current problem fields and fields planted to Cry34/35Ab1 corn during the year the field was sampled (Table 3). Both parameters were positively correlated with abundance of *D. v. virgifera*. Forty-four percent of the abundance of *D. barberi* was explained in multiple regression

analysis (Table 3), and parameters retained in the model were continuous cornfields and years planted to corn continuously. Continuous cornfields were positively correlated with abundance of *D. barberi*, but abundance negatively correlated with the number of years corn was continuously planted in a field. Multiple regression analysis of field history data explained 54% of the variation in the ratio of *D. v. virgifera* to *D. barberi* and both current problem fields and years planted to corn continuously were significantly and positively correlated with the ratio of *D. v. virgifera* to *D. barberi* (Table 3).

Significant differences among field types were present for field histories (Table 4). The proportion of years planted to corn during the time investigated (2003-2013 or 2014) was significantly lower for recently rotated fields compared the other field types. Crop rotations in recently rotated fields varied in length from three-year rotation schemes with one year planted to a non-corn crop (i.e., two years of corn followed by soybean (*Glycine max* (L.) Merr.) to seven-year rotation with five consecutive years of non-corn crops (i.e., corn planted for two years, alfalfa *Medicago sativa* L. planted for four years and one year of oats *Avena sativa* L.) (Supp. Table S1). Soybean was the only other crop planted in all continuous cornfields and past problem fields and the only other crop in seven of the nine current problem fields (Supp. Tables S2, S3 and S4). The proportion of years planted to corn continuously was significantly lower in recently rotated fields, which was expected given the criteria for selecting those fields, but did not differ among continuous cornfields, past problem fields and current problem fields (Table 4).

Management of *Diabrotica* spp. differed significantly among field types. The use of non-Bt corn differed significantly among field types with recently rotated fields planted to non-Bt corn more often than past problem fields, which were almost never planted to non-Bt corn (Table 4). Furthermore, when fields were planted to corn, significantly more Cry3Bb1 corn was planted

in past problem fields compared to Cry3Bb1 corn planted in recently rotated fields (Table 4). Continuous cornfields and current problem fields did not differ from any other field type in either the planting of non-Bt corn or Cry3Bb1 corn. Planting of Cry34/35Ab1, mCry3A, or Cry3Bb1 and Cry34/35Ab1 pyramided corn did not differ among field types (Table 4). No fields sampled contained corn producing eCry3.1Ab toxin. Use of soil-applied insecticide also differed significantly among field types (Table 4), and was significantly lower in recently rotated fields and current problem fields compared to continuous cornfields. Use of foliar insecticides on corn was very rare, and did not differ among field types (Table 4).

Field histories of past problem fields differed significantly between the time before and after greater than expected injury to Bt corn was observed (Table 5). The use of Cry3Bb1 corn in past problem fields was significantly higher preceding greater than expected injury compared to afterward. The amount of corn planted continuously in past problem fields was significantly greater after versus before severe injury to Bt corn was observed. Additionally, after greater than expected injury, farmers planted more pyramided Cry3Bb1 and Cry34/35Ab1 corn and applied soil insecticide more often.

Discussion

We sampled 47 cornfields in Iowa during 2013 and 2014 and found significantly greater root injury (Fig. 2) and abundance of *D. v. virgifera* (Fig. 3) in current problem fields compared to recently rotated fields, continuous cornfields and past problem fields. Although root injury, abundance of *D. v. virgifera* and abundance of *D. barberi* (Fig. 4) did not differ among recently rotated fields, continuous cornfields and past problem fields, inputs for management of *Diabrotica* spp. in recently rotated fields were significantly lower in terms of the amount of Cry3Bb1 corn planted and the application of a soil insecticide (Table 4). Additionally, field

histories were similar between continuous cornfields and current problem fields with the exception of significantly greater use of soil-applied insecticides in continuous cornfields. This result implies that the addition of soil-applied insecticides may have prevented continuous cornfields from becoming current problem fields. In past problem fields, farmers mitigated the risk of injury from potentially Bt-resistant *D. v. virgifera* populations by planting more pyramided Bt corn and increasing the use of soil-applied insecticides (Table 5).

Previous studies have characterized root injury from cornfields with unexpected injury to Bt corn (Gassmann et al. 2011, 2012, 2014; Wangila et al. 2015), which were similar to the level of root injury observed here in current problem fields (Fig. 2). We sampled adult *D. v. virgifera* and *D. barberi* from current problem fields and found that peak abundance of *D. v. virgifera* alone exceeded the traditional economic threshold of 6 *Diabrotica* spp. adults captured per sticky trap per day (Hein and Tollefson 1985) on average by fivefold and fourfold during 2013 and 2014, respectively (Fig. 3). Field histories from current problem fields illustrated a pattern of relying primarily on Cry3Bb1 corn for management of *Diabrotica* spp. with no applications of conventional insecticides and low instances of crop rotation (Table 4, Supp. Table S4), again similar to other studies (Gassmann et al. 2011, 2012, 2014; Wangila et al. 2015). Laboratory strains of *D. v. virgifera* have been selected for resistance to Cry3Bb1 corn, with resistance developing in as few as three generations of selection (Meihls et al. 2008). Other laboratory strains of *D. v. virgifera* have also been selected for resistance to Cry34/35Ab1 (Lefko et al. 2008, Deitloff et al. 2015), mCry3A (Meihls et al. 2011) and eCry3.1Ab (Frank et al. 2013) Bt toxins, and resistance can develop within three to eight generations. Although populations of *D. v. virgifera* from current problem fields were not screened for Bt resistance as part of this study, considering the evidence that Bt resistance can be rapidly selected for and the similarity in field

history with previously sampled fields with Bt-resistant populations, suggests that *D. v. virgifera* from current problem fields may have been Bt resistant.

We hypothesized that, following greater than expected injury to Cry3 corn, farmers would mitigate the risk of future root injury by either rotating fields out of corn production, changing the type of Bt corn planted, applying more conventional insecticides or some combination of these approaches. We found that farmers of past problem fields grew significantly more continuous corn after experiencing greater than expected injury to Cry3 corn, indicating that increased crop rotation was not used to mitigate the risk of future *Diabrotica* spp. injury. However, following greater than expected injury, farmers grew significantly more Cry3Bb1 and Cry34/35Ab1 pyramided corn, applied soil-insecticides more frequently, and grew corn solely expressing Cry3Bb1 significantly less (Table 5). These data indicate that farmers have typically mitigated instances of greater than expected injury to Bt corn by primarily growing corn pyramided with Cry3Bb1 and Cry34/35Ab1 either alone or with soil-applied insecticide.

Field histories were similar among continuous cornfields and current problem fields (Table 4, Supp. Tables S2 and S4), with the exception of soil-applied insecticide use in continuous cornfields. This difference suggests that soil-applied insecticides may have been an important factor in preventing the occurrence of greater than expected injury to Bt corn. However, this does not mean that *D. v. virgifera* in continuous cornfields are susceptible to Cry3Bb1 corn. Pyramids of insecticides that target the same pest are effective at delaying resistance when toxins in the pyramid have independent modes of action and mortality is high for homozygous susceptible individuals, but pyramids become less effective at delaying resistance as pest mortality decreases (Comins 1986, Gould 1998, Roush 1998, Tabashnik and

Gould 2012). Pyramiding soil-applied insecticides with Bt corn may be detrimental for resistance management of *Diabrotica* spp. because soil insecticides typically do not kill enough larvae to delay resistance to Bt corn (Petzold-Maxwell et al. 2013). Furthermore, field experiments have demonstrated that combining soil-applied insecticide with Bt corn delays adult emergence (Petzold-Maxwell et al. 2013, Frank et al. 2015), which can promote assortative mating of Bt-selected individuals leading to more rapid Bt resistance evolution (Gould 1998). While use of insecticides may have reduced the risk of greater than expected injury, it is currently unknown to what extent insecticides will affect the rate of resistance development.

Integrated Pest Management can reduce the need for farmers to invest in pest management inputs. Root injury and the abundance of *D. v. virgifera* did not differ among recently rotated fields, continuous cornfields and past problem fields. However, recently rotated fields planted significantly less Bt corn and used significantly less soil-applied insecticide (Table 4). Furthermore, IPM can complement resistance management by diversifying the causes of pest mortality (McGaughey and Whalon 1992). Although increasing the use of crop rotation would diversify the causes of *Diabrotica* spp. mortality compared to only relying on Bt corn, incorporating crop rotation alone may not be the optimal strategy for delaying Bt resistance. There are areas of the Corn Belt where both Bt-resistant and rotation-resistant *D. v. virgifera* already co-occur (Gray et al. 2009, Gray 2014). Models analyzing the predicted interactions among Bt resistance, rotation resistance and *D. v. virgifera* indicate that in areas where rotation resistance is absent, like Iowa (Dunbar and Gassmann 2013), inheritance of Bt resistance is the most important factor affecting the development of Bt resistance, regardless of the area planted to continuous corn (Crowder et al. 2005). Where rotation-resistant *D. v. virgifera* is present or thought to soon become an issue, planting first-year corn that produce Bt toxins can delay

resistance to both Bt corn and crop rotation (Crowder et al. 2005). Additional key components of IPM include the use of economic thresholds and scouting to determine the need for pest management (Pedigo and Rice 2006). Although adult abundance on average exceeded the economic threshold in all field types, some individual fields had peak abundances below economic thresholds (Supp. Table S5) suggesting that management of *Diabrotica* spp. would not be needed in those fields if planted to corn the following year. However, it is difficult to predict larval injury based on adult abundance, which means economic thresholds for *Diabrotica* spp. may be of limited value (Foster et al. 1986). Furthermore, a survey of Iowa farmers has shown that less than half scout for adult *Diabrotica* spp. (Arbuckle 2013).

Competition among closely related species that occupy similar ecological niches can result in species displacement (Reitz and Trumble 2002). Evidence for competition was observed in previously sampled Iowa cornfields that were dominated by either *D. barberi* or *D. v. virgifera* (Dunbar and Gassmann 2013). Although both *D. barberi* and *D. v. virgifera* were captured by sticky traps from all 47 cornfields in this study, the average ratio of *D. v. virgifera* to *D. barberi* in recently rotated cornfields was lower compared to the other field types, especially compared to current problem fields (Table 2). There are two factors that may explain these observations. The first is that Iowa has rotation-resistant *D. barberi* but not rotation-resistant *D. v. virgifera* (Levine and Oloumi-Sadeghi 1991, Dunbar and Gassmann 2013). Previous studies conducted in other areas of the Corn Belt with rotation-resistant *D. barberi*, but without rotation-resistant *D. v. virgifera*, have documented greater prevalence of *D. barberi* in areas where there is a greater frequency of crop rotation (Hill and Mayo 1980). Secondly, *D. barberi* is an inferior competitor to *D. v. virgifera*. Greenhouse studies of intraspecific and interspecific competition among varying infestations rates of each species have shown that *D. v. virgifera* survival is affected

more by intraspecific competition and *D. barberi* survival is affected by both intraspecific and interspecific competition (Piedrahita et al. 1985; Woodson 1993, 1994). In landscapes dominated by continuous cornfields, *D. v. virgifera* have been observed to displace *D. barberi* (Hill and Mayo 1980). *Diabrotica* populations in current problem fields were composed predominantly of *D. v. virgifera*. This likely arose because Bt resistance permitted the survival of *D. v. virgifera*, and increasing populations of *D. v. virgifera* displaced the inferior competitor *D. barberi*.

Diversifying management of *Diabrotica* spp. by incorporating IPM tactics such as crop rotation with the judicious use Bt corn and conventional insecticides can decrease yield loss from *Diabrotica* spp. Data presented here showed that low root injury and low abundance of adult *Diabrotica* spp. in recently rotated fields required significantly fewer management inputs compared to continuous cornfields and past problem fields. Furthermore, severe root injury and large populations of *D. v. virgifera* were associated with fields that lacked management diversity. As with many pest species, utilizing a diverse set of pest management practices likely offers the most sustainable approach for management of *Diabrotica* spp.

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Tables

Table 1. *Diabrotica* spp. management by field type in 2013 and 2014

# Fields (# Fields with Soil Insecticide)						
Field Types ¹	N	Non-Bt	Cry3Bb1	Cry34/35Ab1	mCry3A	Cry3Bb1 + Cry34/35Ab1
2013						
Recently Rotated Field	5	1 (1)	0 (0)	2 (0)	1 (0)	1 (0)
Continuous Cornfield	5	1 (1)	1 (1)	3 (1)	0 (0)	0 (0)
Past Problem Field	5	0 (0)	0 (0)	0 (0)	0 (0)	5 (2)
Current Problem Field	5	0 (0)	5 (0)	0 (0)	0 (0)	0 (0)
2014						
Recently Rotated Field	7	1 (0)	0 (0)	2 (0)	0 (0)	4 (0)
Continuous Cornfield	8	1 (0)	1 (1)	0 (0)	0 (0)	6 (1)
Past Problem Field	8	1 (0)	1 (1)	0 (0)	0 (0)	6 (3)
Current Problem Field	4	0 (0)	2 (0)	0 (0)	2 (0)	0 (0)

¹ Field types sampled in 2013 and 2014: Recently Rotated Field = 2nd year cornfields, Continuous Cornfield = fields planted continuously to corn for > 7 years, Past Problem Field = cornfields with a history of greater than expected injury (> 1 node injured) to Bt corn, Current Problem Field = cornfields currently experiencing greater than 1 root node of injury to Bt corn during the year sampled.

Table 2. Ratio of *Diabrotica v. virgifera* to *D. barberi* captured by sticky traps by field type in 2013 and 2014

Field Types ¹	Ratio <i>D. v. virgifera</i> to <i>D. barberi</i>	
	2013 ²	2014
Recently Rotated Field	10.6 ± 7.3b	0.9 ± 0.4b
Continuous Cornfield	16.2 ± 6.0b	11.9 ± 6.3ab
Past Problem Field	57.7 ± 30.2b	9.3 ± 4.6ab
Current Problem Field	1,562.3 ± 859.9a	26.1 ± 12.6a

¹ Field types sampled in 2013 and 2014: Recently Rotated Field = 2nd year cornfields, Continuous Cornfield = fields planted continuously to corn for > 7 years, Past Problem Field = cornfields with a history of greater than expected injury (> 1 node injured) to Bt corn, Current Problem Field = cornfields currently experiencing greater than 1 root node of injury to Bt corn during the year sampled.

² Letters denote significant differences among field types within year sampled.

Table 3. Multiple linear regression for root injury, abundance of *D. v. virgifera*, abundance of *D. barberi* and the ratio of *D. v. virgifera* to *D. barberi*

Dependent Variable	Parameters	Slope	SE	F	P	Model r^2
Root Injury						0.59
	Current Problem Fields	1.125	0.229	24.46	<0.0001	
	(Intercept)	-0.855	0.142	36.33	<0.0001	
Abundance of <i>D. v. virgifera</i>						0.51
	Current Problem Fields	1.543	0.278	30.70	<0.0001	
	Planted to Cry34/35Ab1 Corn during year sampled	0.842	0.319	6.98	0.013	
	(Intercept)	-0.059	0.132	0.20	0.66	
Abundance of <i>D. barberi</i>						0.44
	Continuous Cornfields	0.545	0.197	7.62	0.010	
	Years of Continuous Corn	-1.238	0.279	19.65	0.0001	
	(Intercept)	-0.052	0.164	0.10	0.75	
Ratio of <i>D. v. virgifera</i> to <i>D. barberi</i>						0.54
	Current Problem Fields	1.473	0.332	19.66	0.0001	
	Years of Continuous Corn	1.394	0.441	9.98	0.003	
	(Intercept)	-0.089	0.258	0.12	0.73	

Table 4. Analysis of variance among field types for various management approaches and cropping history

Cropping History Factor ¹	<i>F</i>	df	<i>P</i>	Field Types ^{2,3}			
				Rotated	Continuous	Past Problem	Current Problem
Planted to Corn	19.22	3, 33	<0.0001	0.49 ± 0.05b	0.94 ± 0.02a	0.87 ± 0.04a	0.77 ± 0.08a
Planted to Continuous Corn	14.18	3, 33	<0.0001	0.17 ± 0.01b	0.84 ± 0.06a	0.66 ± 0.09a	0.55 ± 0.13a
Planted to Non-Bt Corn	4.31	3, 33	0.011	0.61 ± 0.12a	0.30 ± 0.11ab	0.10 ± 0.06b	0.34 ± 0.11ab
Cry3Bb1 Corn	4.58	3, 33	0.009	0.22 ± 0.11b	0.45 ± 0.12ab	0.73 ± 0.07a	0.60 ± 0.12ab
Cry34/35Ab1 Corn	2.66	3, 33	0.06	0.05 ± 0.03	0.13 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
mCry3A Corn	1.09	3, 33	0.37	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.03
Cry3Bb1 + Cry34/35Ab1 Corn	1.91	3, 33	0.15	0.13 ± 0.05	0.12 ± 0.04	0.17 ± 0.03	0.03 ± 0.02
Soil Insecticide Applied	4.59	3, 33	0.009	0.02 ± 0.02c	0.31 ± 0.10ab	0.14 ± 0.05bc	0.00 ± 0.00c
Foliar Insecticide Applied	2.35	3, 33	0.09	0.00 ± 0.00	0.06 ± 0.02	0.03 ± 0.02	0.00 ± 0.00

¹ Data presented on the proportion of total years each management approach was used in a field, with data presented as mean ± standard error of the mean.

² Field types sampled in 2013 and 2014: Recently Rotated Field = 2nd year cornfields, Continuous Cornfield = fields planted continuously to corn for > 7 years, Past Problem Field = cornfields with a history of greater than expected injury (> 1 node injured) to Bt corn, Current Problem Field = cornfields currently experiencing greater than 1 root node of injury to Bt corn during the year sampled.

³ Letters denote significant differences among field types within year sampled.

Table 5. Paired *t*-test comparing management approaches in fields before and after greater than expected injury to Bt corn

Cropping History Factor	<i>t</i>	df	<i>P</i>	Past Problem Fields ¹	
				Before Injury	After Injury
Planted to Corn	2.14	9	0.06	0.86 ± 0.05	0.98 ± 0.02
Planted to Continuous Corn	2.93	9	0.017	0.66 ± 0.10	0.98 ± 0.02
Planted to Non-Bt Corn	1.50	9	0.17	0.11 ± 0.08	0.00 ± 0.00
Cry3Bb1 Corn	5.33	9	0.0005	0.87 ± 0.07	0.25 ± 0.12
Cry34/35Ab1 Corn	.	.	.	0.00 ± 0.00	0.00 ± 0.00
mCry3A Corn	1.00	9	0.34	0.00 ± 0.00	0.01 ± 0.01
Cry3Bb1 + Cry34/35Ab1 Corn	6.55	9	0.0001	0.00 ± 0.00	0.75 ± 0.12
Soil Insecticide Applied	2.77	9	0.022	0.03 ± 0.02	0.36 ± 0.12
Foliar Insecticide Applied	0.50	9	0.63	0.03 ± 0.02	0.05 ± 0.05

¹ Data presented on the proportion of total years each management approach was used in a field, with data presented as mean ± standard error of the mean.

Figures

Figure 1. Distribution of cornfields sampled within Iowa in 2013 and 2014. Location of each field is accurate to the level of county.

Figure 2. Root injury by field type for 2013 and 2014. Root injury was scored on the 0 to 3 node-injury scale (Oleson et al. 2005). Bar heights represent sample means and error bars are the standard error of the mean. Bars with diagonal lines represent root injury data collected in 2013 and bars with solid fill represent data collected in 2014. Differing bar colors represent each field type. Capital letters denote significant differences among field types in 2013 and lowercase letters represent significant differences among field types in 2014.

Figure 3. Mean peak abundance of *D. v. virgifera* capture per trap per day by field type for 2013 and 2014. Bar heights represent sample means and error bars are the standard error of the mean. Bars with diagonal lines represent *D. v. virgifera* captured in 2013 and bars with solid fill represent *D. v. virgifera* captured in 2014. Differing bar colors represent each field type. Capital letters denote significant differences among field types in 2013 and lowercase letters represent significant differences among field types in 2014.

Figure 4. Mean peak abundance of *D. barberi* capture per trap per day by field type for 2013 and 2014. Bar heights represent sample means and error bars are the standard error of the mean. Bars with diagonal lines represent *D. barberi* captured in 2013 and bars with solid fill represent *D. barberi* captured in 2014. Differing bar colors represent each field type. Capital letters denote significant differences among field types in 2013.

Figure 1.

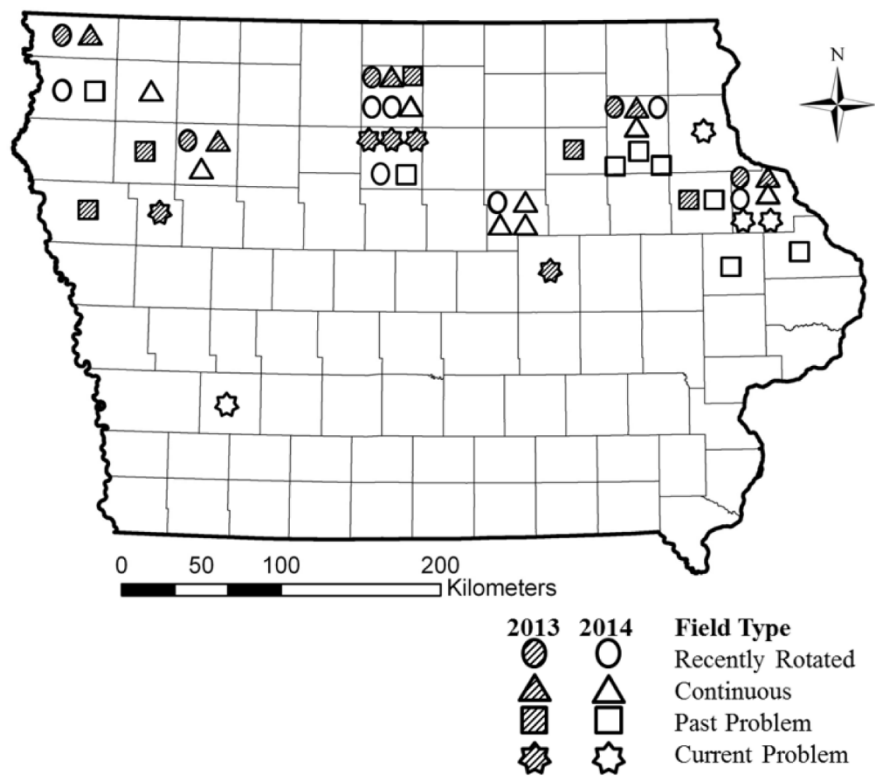


Figure 2.

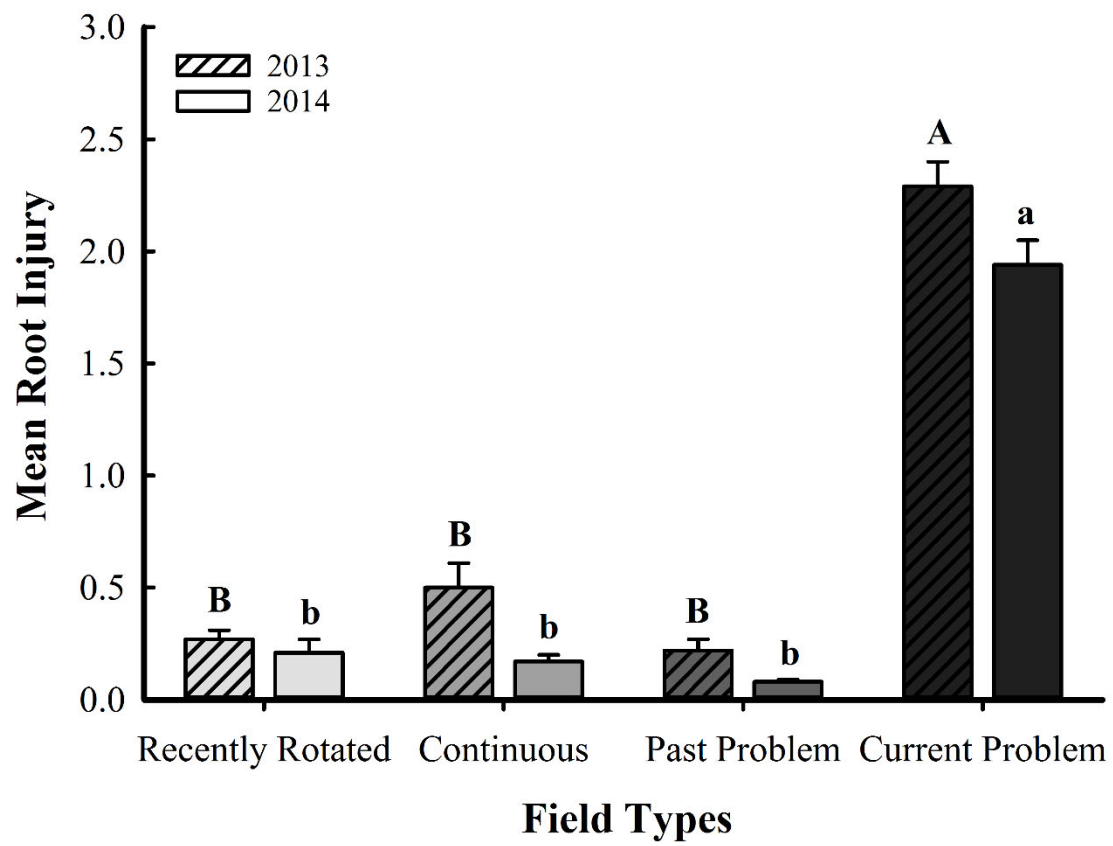


Figure 3.

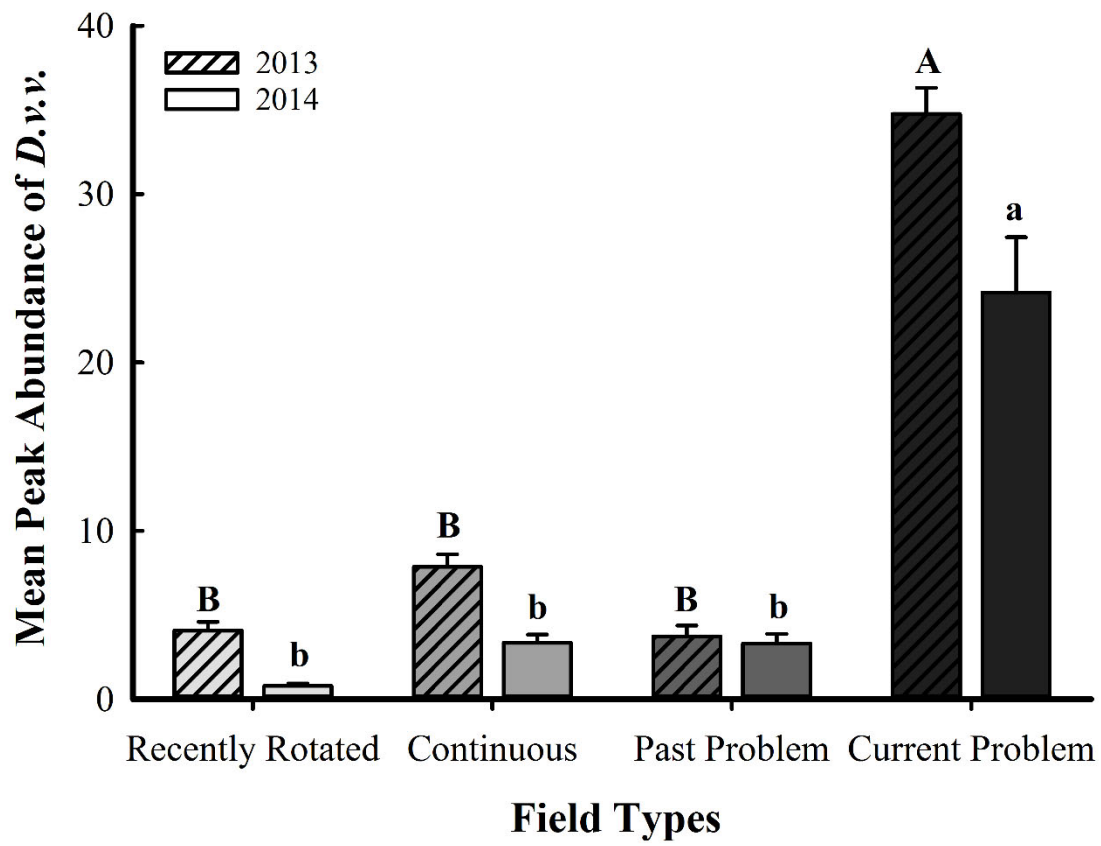
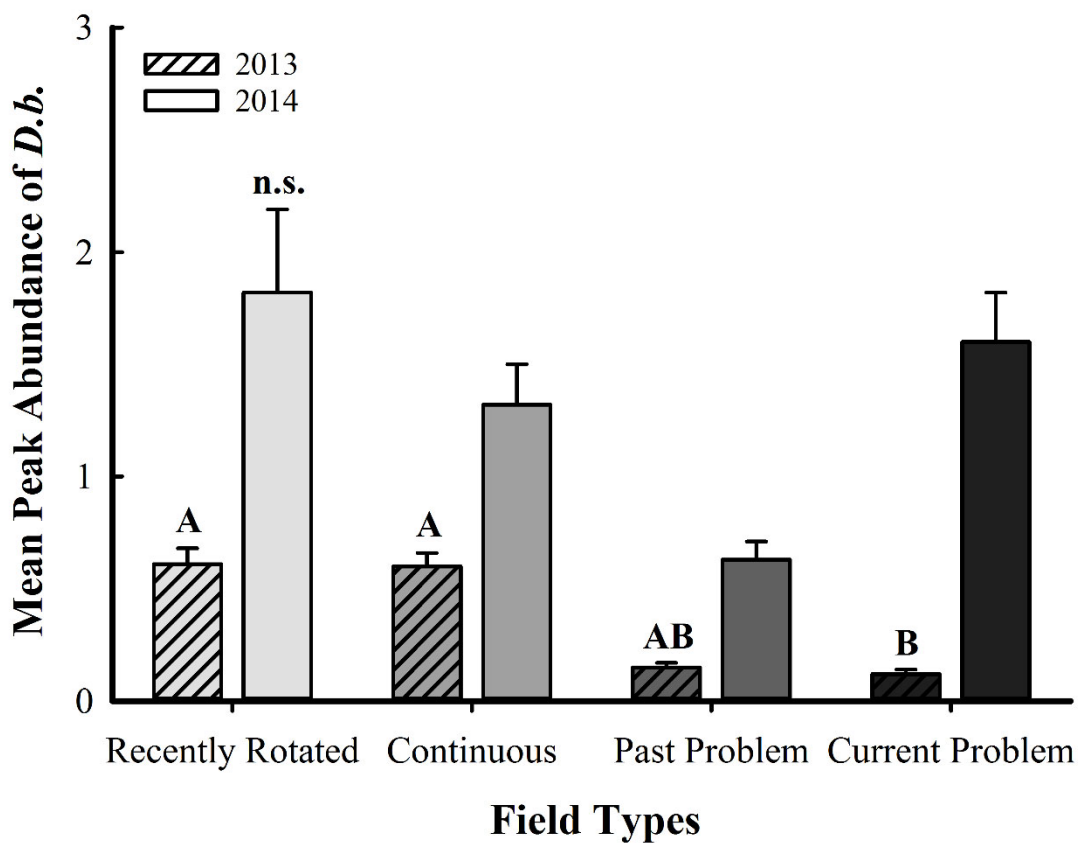


Figure 4.



Supplemental Materials

Supplemental Table S1. Field history of recently rotated fields sampled in 2013 and 2014

	Sampled	2014	2014	2014	2014	2014	2014	2014	2013	2013	2013	2013	2013
Year	Field #	1	2	3	4	5	6 ⁵	7 ⁵	8	9 ⁵	10	11	12
2014	Crop ¹	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin ²	0	4	4	4	4	2	2
	Soil ³	None	None	None	None	None	None	None
	Foliar ⁴	None	None	None	None	None	None	None
2013	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	0	0	1	0	Uk	Uk	Uk	0	3	4	2	2
	Soil	None	None	None	None	Uk	Uk	Uk	Yes	None	None	None	None
	Foliar	None	None	None	None	Uk	Uk	Uk	None	None	None	None	None
2012	Crop	Alfa	Alfa	Soy	Soy	Alfa	Soy	Soy	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	0	Uk	4	0	0
	Soil	None	Uk	None	None	None
	Foliar	None	Uk	None	None	None
2011	Crop	Alfa	Alfa	Corn	Corn	Alfa	Corn	Corn	Soy	Soy	Soy	Alfa	Alfa
	Bt Toxin	.	.	1	1	.	Uk	Uk
	Soil	.	.	None	None	.	Uk	Uk
	Foliar	.	.	None	None	.	Uk	Uk
2010	Crop	Alfa	Alfa	Soy	Corn	Alfa	Soy	Soy	Corn	Corn	Corn	Alfa	Alfa
	Bt Toxin	.	.	.	0	.	.	.	1	Uk	4	.	.
	Soil	.	.	.	None	.	.	.	None	Uk	None	.	.
	Foliar	.	.	.	None	.	.	.	None	Uk	None	.	.
2009	Crop	Oats	Oats	Corn	Soy	Alfa	Corn	Corn	Soy	Soy	Soy	Alfa	Alfa
	Bt Toxin	.	.	1	.	.	Uk	Uk
	Soil	.	.	None	.	.	Uk	Uk
	Foliar	.	.	None	.	.	Uk	Uk
2008	Crop	Corn	Corn	Soy	Corn	Oats	Soy	Corn	Corn	Corn	Corn	Oats	Alfa
	Bt Toxin	0	0	.	1	.	.	Uk	0	Uk	1	.	.
	Soil	None	None	.	None	.	.	Uk	None	Uk	None	.	.
	Foliar	None	None	.	None	.	.	Uk	None	Uk	None	.	.
2007	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Soy	Soy	Corn	Corn	Oats
	Bt Toxin	0	0	1	0	0	Uk	Uk	.	.	Uk	0	.
	Soil	None	None	None	None	None	Uk	Uk	.	.	Uk	None	.
	Foliar	None	None	None	None	None	Uk	Uk	.	.	Uk	None	.

¹ Crop planted: Corn = Corn, Soy = Soybean; Oats = Oats; Alfa = Alfalfa; Sod = Sod, Uk = unknown.

² Rootworm active Bt corn: 0 = no rootworm active Bt, 1 = Cry3Bb1, 2 = Cry34/35Ab1, 3 = mCry3A, 4 = Cry3Bb1 & Cry34/35Ab1, Uk = unknown.

³ Soil-applied insecticide used, Uk = unknown.

⁴ Foliar-applied insecticide used, Uk = unknown.

⁵ Field excluded from cropping history analyses.

Supplemental Table S1 (Continued). Field history of recently rotated fields sampled in 2013 and 2014

Year	Sampled Field #	2014 1	2014 2	2014 3	2014 4	2014 5	2014 6 ⁵	2014 7 ⁵	2013 8	2013 9 ⁵	2013 10	2013 11	2013 12
2006	Crop ¹	Alfa	Alfa	Soy	Soy	Corn	Soy	Soy	Corn	Corn	Soy	Soy	Corn
	Bt Toxin ²	Uk	.	.	0	Uk	.	.	0
	Soil ³	Uk	.	.	None	Uk	.	.	None
	Foliar ⁴	Uk	.	.	None	Uk	.	.	None
2005	Crop	Alfa	Alfa	Corn	Corn	Soy	Corn	Corn	Soy	Corn	Corn	Corn	Corn
	Bt Toxin	.	.	1	1	.	Uk	Uk	.	Uk	1	0	0
	Soil	.	.	None	None	.	Uk	Uk	.	Uk	None	None	None
	Foliar	.	.	None	None	.	Uk	Uk	.	Uk	None	None	None
2004	Crop	Alfa	Alfa	Soy	Corn	Corn	Soy	Soy	Corn	Corn	Soy	Soy	Alfa
	Bt Toxin	.	.	.	0	Uk	.	.	Uk	Uk	.	.	.
	Soil	.	.	.	None	Uk	.	.	Uk	Uk	.	.	.
	Foliar	.	.	.	None	Uk	.	.	Uk	Uk	.	.	.
2003	Crop	Oats	Oats	Corn	Soy	Alfa	Corn	Corn	Corn	Soy	Corn	Corn	Alfa
	Bt Toxin	.	.	1	.	.	Uk	Uk	Uk	.	1	0	.
	Soil	.	.	None	.	.	Uk	Uk	Uk	.	None	None	.
	Foliar	.	.	None	.	.	Uk	Uk	Uk	.	None	None	.

¹ Crop planted: Corn = Corn, Soy = Soybean; Oats = Oats; Alfa = Alfalfa; Sod = Sod, Uk = unknown.

² Rootworm active Bt corn: 0 = no rootworm active Bt, 1 = Cry3Bb1, 2 = Cry34/35Ab1, 3 = mCry3A, 4 = Cry3Bb1 & Cry34/35Ab1, Uk = unknown.

³ Soil-applied insecticide used, Uk = unknown.

⁴ Foliar-applied insecticide used, Uk = unknown.

⁵ Field excluded from cropping history analyses.

Supplemental Table S2. Field history of continuous cornfields sampled in 2013 and 2014

Year	Sampled Field #	2014 1	2014 2	2014 3	2014 4	2014 5	2014 6	2014 7 ⁵	2014 8 ⁵	2013 9	2013 10	2013 11	2013 12	2013 13
2014	Crop ¹	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin ²	1	4	4	4	4	4	4	0
	Soil ³	Yes	None	Yes	None	None	None	None	Uk
	Foliar ⁴	Yes	None	None	None	None	None	None	Uk
2013	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	2	2	4	2	4	4	Uk	Uk	2	0	1	2	2
	Soil	None	None	Yes	None	None	None	Uk	Uk	Yes	Yes	Yes	None	None
	Foliar	None	None	None	None	None	None	Uk	Uk	None	None	None	None	None
2012	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	None	1	4	1	0	0	Uk	Uk	None	4	1	4	1
	Soil	Yes	Yes	None	None	Yes	Yes	Uk	Uk	Yes	None	None	None	None
	Foliar	None	Yes	Yes	None	None	None	Uk	Uk	None	Yes	Yes	None	None
2011	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	2	4	1	0	0	Uk	Uk	0	4	1	2	1
	Soil	None	None	None	None	Yes	Yes	Uk	Uk	Yes	None	None	None	None
	Foliar	None	None	None	None	None	None	Uk	Uk	None	None	None	None	None
2010	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	2	Yes	4	1	0	0	Uk	Uk	0	1	1	1	1
	Soil	None	Yes	None	None	Yes	Yes	Uk	Uk	None	None	None	None	None
	Foliar	None	Yes	None	None	None	None	Uk	Uk	None	None	None	None	None
2009	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	None	2	1	1	0	0	Uk	Uk	1	1	1	2	1
	Soil	None	None	None	None	Yes	Yes	Uk	Uk	None	None	None	None	None
	Foliar	None	None	None	None	None	None	Uk	Uk	None	None	None	None	None
2008	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	None	1	1	0	0	Uk	Uk	0	1	1	2	1
	Soil	None	None	None	None	Yes	Yes	Uk	Uk	Yes	None	None	None	None
	Foliar	None	None	None	None	None	None	Uk	Uk	None	None	None	None	None
2007	Crop	Soy	Soy	Corn	Corn	Corn	Corn	Soy	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	.	.	1	1	0	0	.	Uk	0	1	1	2	1
	Soil	.	.	None	None	Yes	Yes	.	Uk	Yes	None	None	None	None
	Foliar	.	.	None	None	None	None	.	Uk	None	None	None	None	None

¹ Crop planted: Corn = Corn, Soy = Soybean; Oats = Oats; Alfa = Alfalfa; Sod = Sod, Uk = unknown.² Rootworm active Bt corn: 0 = no rootworm active Bt, 1 = Cry3Bb1, 2 = Cry34/35Ab1, 3 = mCry3A, 4 = Cry3Bb1 & Cry34/35Ab1, Uk = unknown.³ Soil-applied insecticide used, Uk = unknown.⁴ Foliar-applied insecticide used, Uk = unknown.⁵ Field excluded from cropping history analyses.

Supplemental Table S2 (Continued). Field history of continuous cornfields sampled in 2013 and 2014

Year	Sampled Field #	2014 1	2014 2	2014 3	2014 4	2014 5	2014 6	2014 7⁵	2014 8⁵	2013 9	2013 10	2013 11	2013 12	2013 13
2006	Crop¹	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Soy	Corn	Soy	Corn	Corn	Corn
	Bt Toxin²	1	Uk	1	1	0	0	Uk	.	0	.	1	2	1
	Soil³	None	Uk	None	None	Yes	Yes	Uk	.	Yes	.	None	None	None
	Foliar⁴	None	Uk	None	None	None	None	Uk	.	None	.	None	None	None
2005	Crop	Corn	Soy	Soy	Corn	Corn	Corn	Soy	Corn	Corn	Corn	Soy	Corn	Corn
	Bt Toxin	1	.	.	1	0	0	.	Uk	0	1	.	0	1
	Soil	None	.	.	None	Yes	Yes	.	Uk	Yes	None	.	Yes	None
	Foliar	None	.	.	None	None	None	.	Uk	None	None	.	None	None
2004	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Soy	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	Uk	1	1	0	0	Uk	.	Uk	1	1	0	1
	Soil	None	Uk	None	None	Yes	Yes	Uk	.	Uk	None	None	None	None
	Foliar	None	Uk	None	None	None	None	Uk	.	Uk	None	None	None	None
2003	Crop	Corn	Soy	Corn	Corn	Corn	Corn	Soy	Corn	Corn	Corn	Corn	Soy	Corn
	Bt Toxin	1	.	1	1	0	0	.	Uk	Uk	1	1	.	1
	Soil	None	.	None	None	Yes	Yes	.	Uk	Uk	None	None	.	None
	Foliar	None	.	None	None	None	None	.	Uk	Uk	None	None	.	None

¹ Crop planted: Corn = Corn, Soy = Soybean; Oats = Oats; Alfa = Alfalfa; Sod = Sod, Uk = unknown.

² Rootworm active Bt corn: 0 = no rootworm active Bt, 1 = Cry3Bb1, 2 = Cry34/35Ab1, 3 = mCry3A, 4 = Cry3Bb1 & Cry34/35Ab1, Uk = unknown.

³ Soil-applied insecticide used, Uk = unknown.

⁴ Foliar-applied insecticide used, Uk = unknown.

⁵ Field excluded from cropping history analyses.

Supplemental Table S3. Field history of past problem fields sampled in 2013 and 2014

Year	Sampled Field #	2014 1	2014 2 ⁵	2014 3	2014 4	2014 5	2014 6	2014 7 ⁵	2014 8	2013 9 ⁵	2013 10	2013 11	2013 12	2013 13
2014	Crop ¹	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin ²	4	4	4	4	4	4	0	1
	Soil ³	Yes	None	Yes	None	Yes	None	None	Yes
	Foliar ⁴	None	None	None	None	None	None	None	None
2013	Crop	Corn	Corn	Corn	Corn ⁶	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	4	1	4	1	4	1	Uk	1	4	4	4	4	4
	Soil	Yes	Uk	None	None	None	None	Uk	Yes	Uk	None	None	Yes	Yes
	Foliar	None	Uk	None	None	None	None	Uk	None	Uk	None	None	None	None
2012	Crop	Corn	Soy	Corn	Corn	Corn	Corn	Corn	Corn	Corn ⁶	Corn ⁶	Corn	Corn	Corn
	Bt Toxin	1	.	4	1	1	1	Uk	1	1	1	4	4	4
	Soil	None	.	None	None	None	None	Uk	Yes	Uk	None	None	None	Yes
	Foliar	None	.	None	None	None	None	Uk	None	Uk	Yes	None	None	Yes
2011	Crop	Corn	Corn ⁶	Corn	Corn	Corn ⁶	Corn ⁶	Corn ⁶	Corn	Corn	Corn	Corn ⁶	Corn ⁶	Corn ⁶
	Bt Toxin	1	3	4	1	1	3	1	1	1	1	1	1	1
	Soil	None	Uk	None	None	None	None	Uk	Yes	Uk	None	None	None	Yes
	Foliar	None	Uk	None	None	None	None	Uk	None	Uk	None	None	None	Yes
2010	Crop	Corn ⁶	Corn	Soy	Soy	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	1	.	.	1	1	Uk	1	1	1	1	1	1
	Soil	None	Uk	.	.	None	None	Uk	Yes	Uk	None	None	None	Yes
	Foliar	None	Uk	.	.	None	None	Uk	None	Uk	None	None	None	None
2009	Crop	Corn	Corn	Corn ⁶	Corn	Corn	Corn	Corn	Corn ⁶	Corn	Soy	Corn	Corn	Corn
	Bt Toxin	1	Uk	1	0	1	1	Uk	1	1	.	1	1	1
	Soil	None	Uk	None	None	None	None	Uk	None	Uk	.	None	None	None
	Foliar	None	Uk	None	None	None	None	Uk	None	Uk	.	None	None	None
2008	Crop	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	.	1	.	1	.	Uk	1	1	1	1	Uk	1
	Soil	None	.	None	.	None	.	Uk	None	Uk	None	None	Uk	None
	Foliar	None	.	None	.	None	.	Uk	None	Uk	None	None	Uk	None
2007	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Soy	Soy	Corn	Corn	Corn
	Bt Toxin	1	Uk	1	0	1	Uk	Uk	1	.	.	0	1	1
	Soil	None	Uk	None	None	None	Uk	Uk	None	.	.	None	None	None
	Foliar	None	Uk	None	None	None	Uk	Uk	None	.	.	None	None	None

¹ Crop planted: Corn = Corn, Soy = Soybean; Oats = Oats; Alfa = Alfalfa; Sod = Sod, Uk = unknown.

² Rootworm active Bt corn: 0 = no rootworm active Bt, 1 = Cry3Bb1, 2 = Cry34/35Ab1, 3 = mCry3A, 4 = Cry3Bb1 & Cry34/35Ab1, Uk = unknown.

³ Soil-applied insecticide used, Uk = unknown.

⁴ Foliar-applied insecticide used, Uk = unknown.

⁵ Field excluded from cropping history analyses.

⁶ Year cornfield experienced greater than expected injury to Bt corn.

Supplemental Table S3 (Continued). Field history of past problem fields sampled in 2013 and 2014

	Sampled	2014	2014	2014	2014	2014	2014	2014	2014	2013	2013	2013	2013	2013
Year	Field #	1	2 ⁵	3	4	5	6	7 ⁵	8	9 ⁵	10	11	12	13
2006	Crop ¹	Soy	Corn	Corn	Soy	Soy	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin ²	.	Uk	1	.	.	Uk	Uk	1	1	1	0	1	1
	Soil ³	.	Uk	None	.	.	Uk	Uk	None	Uk	None	None	None	None
	Foliar ⁴	.	Uk	None	.	.	Uk	Uk	None	Uk	None	None	None	None
2005	Crop	Corn	Corn	Corn	Corn	Corn	Soy	Corn	Corn	Soy	Soy	Corn	Soy	Corn
	Bt Toxin	1	Uk	1	0	1	.	Uk	1	.	.	0	.	1
	Soil	None	Uk	None	None	None	.	Uk	None	.	.	None	.	None
	Foliar	None	Uk	None	None	None	.	Uk	None	.	.	None	.	None
2004	Crop	Corn	Soy	Corn	Soy	Soy	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	.	1	.	.	Uk	Uk	1	1	Uk	0	Uk	1
	Soil	None	.	None	.	.	Uk	Uk	None	Uk	Uk	None	Uk	None
	Foliar	None	.	None	.	.	Uk	Uk	None	Uk	Uk	None	Uk	None
2003	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Soy	Soy	Corn	Corn	Corn
	Bt Toxin	1	Uk	1	0	1	Uk	Uk	1	.	.	Uk	Uk	1
	Soil	None	Uk	None	None	None	Uk	Uk	None	.	.	Yes	Uk	None
	Foliar	None	Uk	None	None	None	Uk	Uk	None	.	.	None	Uk	None

¹ Crop planted: Corn = Corn, Soy = Soybean; Oats = Oats; Alfa = Alfalfa; Sod = Sod, Uk = unknown.

² Rootworm active Bt corn: 0 = no rootworm active Bt, 1 = Cry3Bb1, 2 = Cry34/35Ab1, 3 = mCry3A, 4 = Cry3Bb1 & Cry34/35Ab1, Uk = unknown.

³ Soil-applied insecticide used, Uk = unknown.

⁴ Foliar-applied insecticide used, Uk = unknown.

⁵ Field excluded from cropping history analyses.

⁶ Year cornfield experienced greater than expected injury to Bt corn.

Supplemental Table S4. Field history of current problem fields sampled in 2013 and 2014

Year	Sampled Field #	2014 1	2014 2	2014 3	2014 4 ⁵	2013 5 ⁵	2013 6	2013 7	2013 8	2013 9
2014	Crop ¹	Corn	Corn	Corn	Corn
	Bt Toxin ²	1	1	3	3
	Soil ³	None	None	None	None
	Foliar ⁴	None	None	None	None
2013	Crop	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	1	1	3	1	1	1	1	1
	Soil	None	None	None	Uk	None	None	None	None	None
	Foliar	None	None	None	Uk	None	None	None	None	None
2012	Crop	Corn	Corn	Soy	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	4	.	3	1	1	4	1	1
	Soil	None	None	.	Uk	None	None	None	None	None
	Foliar	None	None	.	Uk	None	None	None	None	None
2011	Crop	Corn	Soy	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	.	0	Uk	1	1	1	0	1
	Soil	None	.	None	Uk	None	None	None	None	None
	Foliar	None	.	None	Uk	None	None	None	None	None
2010	Crop	Soy	Corn	Sod	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	.	1	.	Uk	Uk	0	1	0	1
	Soil	.	None	.	Uk	Uk	None	None	None	None
	Foliar	.	None	.	Uk	Uk	None	None	None	None
2009	Crop	Corn	Corn	Sod	Corn	Soy	Soy	Corn	Corn	Corn
	Bt Toxin	1	Uk	.	Uk	.	.	1	0	1
	Soil	None	None	.	Uk	.	.	None	None	None
	Foliar	None	None	.	Uk	.	.	None	None	None
2008	Crop	Corn	Corn	Sod	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	Uk	.	Uk	Uk	0	1	0	1
	Soil	None	None	.	Uk	Uk	None	None	None	None
	Foliar	None	None	.	Uk	Uk	None	None	None	None
2007	Crop	Corn	Corn	Sod	Corn	Soy	Soy	Corn	Corn	Corn
	Bt Toxin	1	Uk	.	Uk	.	.	1	Uk	1
	Soil	None	None	.	Uk	.	.	None	Uk	None
	Foliar	None	None	.	Uk	.	.	None	Uk	None

¹ Crop planted: Corn = Corn, Soy = Soybean; Oats = Oats; Alfa = Alfalfa; Sod = Sod, Uk = unknown.

² Rootworm active Bt corn: 0 = no rootworm active Bt, 1 = Cry3Bb1, 2 = Cry34/35Ab1, 3 = mCry3A, 4 = Cry3Bb1 & Cry34/35Ab1, Uk = unknown.

³ Soil-applied insecticide used, Uk = unknown.

⁴ Foliar-applied insecticide used, Uk = unknown.

⁵ Field excluded from cropping history analyses.

Supplemental Table S4 (Continued). Field history of current problem fields sampled in 2013 and 2014

	Sampled	2014	2014	2014	2014	2013	2013	2013	2013	2013
Year	Field #	1	2	3	4 ⁵	5 ⁵	6	7	8	9
2006	Crop ¹	Corn	Soy	Sod	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin ²	1	.	.	Uk	Uk	0	1	Uk	1
	Soil ³	None	.	.	Uk	Uk	None	None	Uk	None
	Foliar ⁴	None	.	.	Uk	Uk	None	None	Uk	None
2005	Crop	Soy	Corn	Sod	Corn	Soy	Soy	Corn	Soy	Corn
	Bt Toxin	.	0	.	Uk	.	.	0	.	1
	Soil	.	None	.	Uk	.	.	None	.	None
	Foliar	.	None	.	Uk	.	.	None	.	None
2004	Crop	Corn	Alfa	Corn	Corn	Corn	Corn	Corn	Corn	Corn
	Bt Toxin	1	.	0	Uk	Uk	0	0	Uk	1
	Soil	None	.	None	Uk	Uk	None	None	Uk	None
	Foliar	None	.	None	Uk	Uk	None	None	Uk	None
2003	Crop	Corn	Alfa	Corn	Corn	Soy	Soy	Corn	Soy	Corn
	Bt Toxin	1	.	0	Uk	.	.	0	.	1
	Soil	None	.	None	Uk	.	.	None	.	None
	Foliar	None	.	None	Uk	.	.	None	.	None

¹ Crop planted: Corn = Corn, Soy = Soybean; Oats = Oats; Alfa = Alfalfa; Sod = Sod, Uk = unknown.

² Rootworm active Bt corn: 0 = no rootworm active Bt, 1 = Cry3Bb1, 2 = Cry34/35Ab1, 3 = mCry3A, 4 = Cry3Bb1 & Cry34/35Ab1, Uk = unknown.

³ Soil-applied insecticide used, Uk = unknown.

⁴ Foliar-applied insecticide used, Uk = unknown.

⁵ Field excluded from cropping history analyses.

Supplemental Table S5. Root injury, mean peak abundance of *D. v. virgifera*, mean peak abundance of *D. barberi*, and the ratio of *D. v. virgifera* to *D. barberi* for all individual fields by field type sampled in 2013 and 2014

Field Type	Year		Root Injury ¹	Peak Abundance of <i>Diabrotica</i> spp. ²		
	Sampled	Field #		<i>D. v. virgifera</i>	<i>D. barberi</i>	Ratio <i>D.v.v.</i> to <i>D.b.</i> ³
Recently Rotated Fields	2014	1	0.02 ± 0.01	0.02 ± 0.02	0.18 ± 0.08	0.1 : 1
		2	0.04 ± 0.01	0.42 ± 0.08	0.52 ± 0.11	1.1 : 1
		3	0.06 ± 0.01	0.29 ± 0.07	0.14 ± 0.07	2.4 : 1
		4	0.02 ± 0.01	1.03 ± 0.17	0.60 ± 0.14	1.9 : 1
		5	1.07 ± 0.35	3.20 ± 0.56	7.93 ± 1.56	0.4 : 1
		6 ⁴	0.05 ± 0.02	0.12 ± 0.05	0.73 ± 0.23	0.1 : 1
		7 ⁴	0.22 ± 0.09	0.39 ± 0.10	2.67 ± 0.41	0.1 : 1
	2013	8	0.20 ± 0.05	5.74 ± 0.61	1.17 ± 0.16	4.5 : 1
		9 ⁴	0.33 ± 0.11	0.68 ± 0.20	0.17 ± 0.05	4.5 : 1
		10	0.19 ± 0.08	2.03 ± 0.32	1.05 ± 0.12	1.5 : 1
		11	0.46 ± 0.14	10.14 ± 0.94	0.24 ± 0.08	39.7 : 1
		12	0.16 ± 0.08	1.79 ± 0.13	0.44 ± 0.08	2.8 : 1
Continuous Cornfields	2014	1	0.28 ± 0.07	14.81 ± 0.83	1.75 ± 0.25	10.5 : 1
		2	0.14 ± 0.04	1.43 ± 0.29	2.47 ± 0.31	0.6 : 1
		3	0.04 ± 0.01	0.17 ± 0.05	0.27 ± 0.11	0.7 : 1
		4	0.12 ± 0.04	1.21 ± 0.28	0.04 ± 0.02	38.2 : 1
		5	0.11 ± 0.07	0.03 ± 0.02	0.19 ± 0.06	0.2 : 1
		6	0.13 ± 0.04	2.60 ± 0.38	0.08 ± 0.03	42.3 : 1
		7 ⁴	0.17 ± 0.05	3.87 ± 0.88	2.94 ± 0.98	1.8 : 1
		8 ⁴	0.41 ± 0.14	2.60 ± 0.34	2.92 ± 0.26	1.0 : 1
	2013	9	0.05 ± 0.02	7.26 ± 1.16	0.56 ± 0.07	17.1 : 1
		10	2.11 ± 0.21	13.19 ± 1.38	0.83 ± 0.19	16.7 : 1
		11	0.14 ± 0.05	1.41 ± 0.17	0.83 ± 0.13	2.1 : 1
		12	0.28 ± 0.10	12.37 ± 1.44	0.27 ± 0.07	37.5 : 1
		13	0.08 ± 0.02	5.07 ± 0.58	0.50 ± 0.08	7.8 : 1
Past Problem Fields	2014	1	0.02 ± 0.01	0.15 ± 0.04	0.10 ± 0.03	2.1 : 1
		2 ⁴	0.06 ± 0.04	1.57 ± 0.22	0.22 ± 0.06	7.2 : 1
		3	0.04 ± 0.01	0.38 ± 0.05	2.06 ± 0.29	0.2 : 1
		4	0.08 ± 0.02	0.45 ± 0.10	0.28 ± 0.07	2.4 : 1
		5	0.03 ± 0.01	0.29 ± 0.06	0.24 ± 0.05	1.6 : 1
		6	0.22 ± 0.06	12.78 ± 1.76	0.47 ± 0.16	30.1 : 1
		7 ⁴	0.08 ± 0.04	1.57 ± 0.20	1.33 ± 0.15	1.3 : 1
		8	0.13 ± 0.06	9.33 ± 1.93	0.32 ± 0.13	2.4 : 1

¹ Mean root injury (± SEM) rated on the 0 to 3 node-injury scale.

² Mean peak abundance (± SEM) of *Diabrotica* spp. capture per trap per day.

³ Ratio of total *D. v. virgifera* to *D. barberi* captured by sticky traps.

⁴ Field excluded from cropping history analyses.

Supplemental Table S5 (Continued). Root injury, mean peak abundance of *D. v. virgifera*, mean peak abundance of *D. barberi*, and the ratio of *D. v. virgifera* to *D. barberi* for all individual fields by field type sampled in 2013 and 2014

Field Type	Year		Root Injury ¹	Peak Abundance of <i>Diabrotica</i> spp. ²		
	Sampled	Field #		<i>D. v. virgifera</i>	<i>D. barberi</i>	Ratio <i>D.v.v.</i> to <i>D.b.</i> ³
Past Problem Fields	2013	9 ⁴	0.19 ± 0.06	0.44 ± 0.11	0.29 ± 0.04	1.4 : 1
		10	0.07 ± 0.04	2.83 ± 0.29	0.03 ± 0.02	135.0 : 1
		11	0.03 ± 0.01	2.41 ± 0.30	0.18 ± 0.04	22.9 : 1
		12	0.41 ± 0.10	12.62 ± 1.35	0.12 ± 0.03	127.1 : 1
		13	0.42 ± 0.20	0.31 ± 0.12	0.12 ± 0.07	1.9 : 1
Current Problem Fields	2014	1	1.49 ± 0.26	13.78 ± 2.23	0.22 ± 0.06	63.0 : 1
		2	2.23 ± 0.16	45.88 ± 4.32	2.70 ± 0.40	17.6 : 1
		3	1.41 ± 0.16	31.02 ± 7.94	2.63 ± 0.42	18.2 : 1
		4 ⁴	2.64 ± 0.10	3.99 ± 0.38	0.70 ± 0.11	5.7 : 1
	2013	5 ⁴	3.00 ± 0.00	34.42 ± 2.11	0.00 ± 0.00	2478.0 : 1
		6	1.73 ± 0.19	36.01 ± 1.16	0.32 ± 0.06	172.1 : 1
		7	2.70 ± 0.22	36.63 ± 4.22	0.01 ± 0.01	4537.0 : 1
		8	2.75 ± 0.13	20.47 ± 2.32	0.13 ± 0.06	153.5 : 1
		9	1.28 ± 0.10	46.29 ± 1.98	0.15 ± 0.05	455.4 : 1

¹ Mean root injury (± SEM) rated on the 0 to 3 node-injury scale.

² Mean peak abundance (± SEM) of *Diabrotica* spp. capture per trap per day.

³ Ratio of total *D. v. virgifera* to *D. barberi* captured by sticky traps.

⁴ Field excluded from cropping history analyses.